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A SIMPLE INEXPENSIVE SLIDE WARMER

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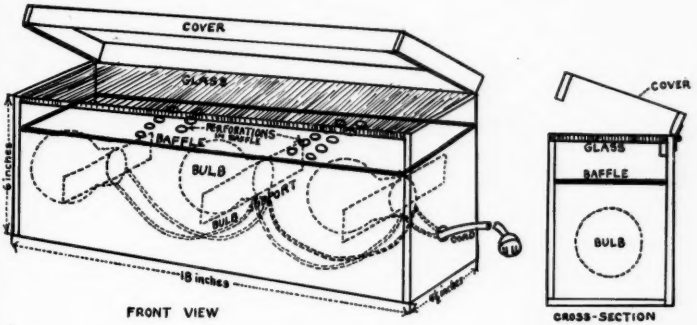
During the past year we have been using with satisfaction a very simple and inexpensive slide warmer. It would probably be of use to others since commercial slide warmers are quite expensive. The cost of this warmer is about one dollar, not including labor.

The obvious difficulties in making an inexpensive home-made slide warmer were to obtain a flat smooth surface easily heated yet not injured by water and to obtain uniform heating. This we accomplished by using a sheet of *glass* on which to place the slides instead of metal and by using a thin strip of cheap metal as a "baffle" to distribute heat more evenly.



The slide warmer is an elongate box of 3-ply wood covered with a sheet of ordinary window glass, on which are placed the slides to be heated. The glass reaches to the front edge of the box (see "Cross section" in figure) so that slides may be slipped off or on with ease. The heat is derived from three evenly spaced 10-watt electric bulbs, arranged parallel and supported by cross bars (bulb-supports) of wood or metal. Just above the bulbs is a sheet of thin galvanized plate (baffle), represented by heavy line in figure, which has been

perforated with a pointed instrument in the regions between the bulbs so as to distribute the heat more evenly. A cover made of strips of wood with a glass (or cellophane) top is provided to keep dust off the slides while they are being heated.

The warmer, as we use it, is 18 inches long and holds about 16 slides. If a larger box is preferred, one 10-watt bulb should be provided for every 6 inches of length. The warmer is 6 inches tall so that it reaches just above the staining jars. It can thus be placed permanently behind the jars and is easily reached.

The temperature of this warmer has been found to range between 41° and 43°C. at various points, as ascertained by laying a thermometer on the glass surface.

Sections made with paraffins having melting points varying from 52°–58°C. spread perfectly, and may be left on the warmer indefinitely without danger of accumulating dust. It is apparent at a glance thru the transparent top of the cover when they are dry. One edge of the cover may be raised to permit the water vapor to escape more rapidly.

A METHOD OF ELIMINATING THE ELECTRIFICATION OF PARAFFIN RIBBONS[1]

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ABSTRACT.—**An apparatus for effectively eliminating the electrification of paraffin ribbons can be constructed for about $2. The essential parts are: a Ford T-TT induction coil to which is connected two, 4-inch brass or copper strips. To the end of one strip is soldered a copper disc 1½ inches in diameter, which is then covered with heavy tinfoil. To the second strip is soldered one-half of a similar disc and its surface is also covered with several layers of tinfoil. The tinfoil should extend ¼ inch beyond the straight edge of the half circle copper plate. The foil extending is cut to present 15 or 20 pointed projections. The coil is supplied with current from a toy train transformer yielding 5 to 12 volts or 4 to 6 amps. When the coil is in operation the two discs should be moved apart to a distance just beyond which the spark fails to jump. The apparatus is so set that the two brass strips are parallel to and 1 or 2 inches above the microtome knife. A bell push-button may be inserted into the circuit so that the operation of the apparatus may be controlled by the foot, leaving the hands free to handle the ribbon.**

One of the most annoying situations encountered in extensive sectioning of paraffin embedded tissue is the electrification of the paraffin ribbon. The static accumulation is often so great that the ribbon adheres to anything brought near it. Under adverse conditions the tendency of ribbon electrification may continue for days making serial work practically impossible and resulting in a great loss of time.

We have been especially confronted by the problem of ribbon electrification in making serial sections of guinea pig embryos. The friction resulting from cutting the tough uterine muscles seemed especially productive of static electricity. We have tried most of the devices recommended to eliminate this difficulty, such as burning a flame near the microtome, boiling water, cooling the surrounding air, humidifying the air with a fine spray of water, grounding the micro-

[1]This investigation was supported by a grant from the Committee for Research in Problems of Sex, National Research Council. Grant administered by Prof. William C. Young.

tome, applying electrical charges from batteries, etc. But these methods, some of which may prove effective for a short time, have failed to be completely satisfactory since no one of them can be depended upon to entirely eliminate electrification.

The following apparatus, which can be constructed at a cost of approximately $2, has been found very effective. It consists primarily of a Ford T-TT induction coil (Fig. 1-A) which may be purchased second-hand from many automobile wrecking establishments for approximately 50c, or new from dealers in auto parts or from mail-order houses for prices ranging from 75–90c. Binding posts may be soldered to the coil as shown in the figure. To the binding posts are

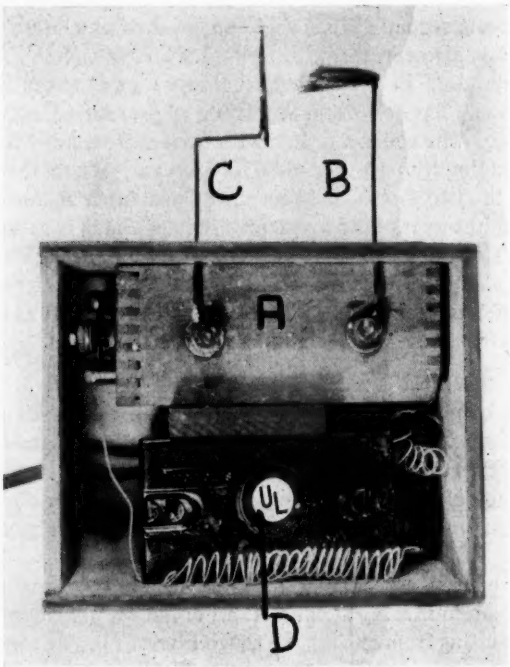

FIG. 1. Top view of apparatus. (A) Ford T-TT induction coil; (B) and (C) brass strips with copper discs; (D) toy train transformer.

connected two, 4-inch brass or copper strips (B & C), or if they are not available sufficiently strong copper wire may be used. To the end of one brass strip (C) is soldered a copper disc 1½ inches in diameter which is then covered with some heavy tinfoil. To the second

strip (B) is soldered one-half of a similar disc and its surface is also covered with several layers of heavy tinfoil. The tinfoil is allowed to extend about ¼ inch beyond the straight edge of the half circle copper plate. The foil extending is now cut so as to present 15 or 20 pointed projections as shown in the illustration. The points of the layers of tinfoil are separated so as to give several rows of projections.

The coil is supplied with current from a transformer (D) of which several types may be used. The one used in the above apparatus is a toy train transformer yielding 5 to 12 volts and 4 to 6 amps. Other transformers such as a radio power transformer or a Lionel multivolt have been found to work successfully; however, a 6 to 11-volt bell transformer failed to work, probably due to the low amperage output (⅓ amp). The transformer is connected directly to the 110-volt A. C. current, and if of the adjustable type should be set to run the coil at 4 to 5 volts. These small transformers may be purchased for approximately $1.15.

When the coil is in operation and the two discs are at a short distance from each other, long sparks will be seen to jump from the disc with the projections to the flat disc. The discs should now be moved apart to a distance just beyond which the spark fails to jump, thus increasing the efficiency of the apparatus, eliminating the unnecessary noise produced by the jumping spark and protecting the sharp projections against burning.

The box is so set that the two brass strips are parallel to and 1 or 2 inches above the microtome knife. It has been found very convenient to insert an ordinary bell push-button into the circuit so that the apparatus may be controlled by the foot thereby leaving the hands free to handle the ribbon. When electrification of the paraffin ribbon occurs one need only press the push-button thereby causing the coil to operate and to ionize the surrounding air. It will be found that the ribbon, no matter how heavily charged, will completely lose its charge within a few seconds.

The author wishes to express his appreciation to Mr. John L. Boling for his many helpful suggestions in building the apparatus.

ALIZARIN RED S TECHNIC APPLIED TO ELASMOBRANCH INTEGUMENT

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While making a study of the scales of the spiny dogfish, *Squalus acanthias*, various types of stains were used.[1] Best results were obtained in material treated with alizarin red S and studied at various times during the dehydration and clearing processes.

The general procedure (applicable to either fresh or preserved material) is as follows: Large pieces of integument are carefully stripped from the body with a minimum of subcutaneous tissue. These are then soaked in water for one to three days, or longer, to loosen most of the adhering connective tissue, which tends to hold the stain. Fresh skin, of course, responds to this treatment more rapidly than does preserved material. During this time all loosened strands are occasionally picked away with forceps. Scraping is avoided as it crushes many of the scale-spines. After most of the connective tissue has been removed, the skin, without preliminary treatment in alkali, is put into a generous amount of 0.4% alizarin red S solution (aqueous).[2]

Altho the length of time in the stain is subject to considerable variation, two to three hours is usually sufficient. The proper stain is reached when weakly pigmented areas are deep pink in color. This progressive method gives more satisfactory results than a regressive one. If, however, a piece is slightly overstained, the excess may be removed by soaking in ammonia solution. We use one part of concentrated ammonium hydroxide and nine parts of water. Unsatisfactory results usually follow attempts to destain skin which has been greatly overstained.

After washing thoroly in water, the skin is transferred thru 50% and 75% alcohol (about 6 hours in each) to 95% where it is left for about 12 hours. It is then cleared in methyl salicylate. The clearing process is more rapid if the skin is thoroly blotted on removal from the alcohol. If the atmosphere is quite damp, blotting saves a change of 95% alcohol and also lessens the time of actual clearing. Frequently, pieces transferred directly from the first 95% alcohol to the salicylate, without blotting, do not clear.

[1]Sayles, L. P. and Hershkowitz, H. G. 1937. Placoid scale types and their distribution in *Squalus acanthias*. Biol. Bull., **73**, 51–66.

[2]Three different lots of this dye were employed in the work, two of them purchased from the National Aniline and Chemical Co.

One serious difficulty encountered with material cleared in salicylate is that it is not flexible. The skin may, however, be kept relatively soft if it is transferred to a mixture of one part fresh 95% alcohol and five parts salicylate. There is some evidence that pieces may clear better in such a mixture. The thin skin of "pups" and the mucous membrane of larger fish, which carry over very little alcohol, seem to clear more readily in the presence of the added fresh alcohol.

For microscopic examination a piece of the skin may be placed in the glass top of a large Petri dish, a little fresh methyl salicylate added, and the bottom part of a somewhat smaller dish set into the top to rest against the skin and flatten it out. If the correct amount of salicylate is used, the skin will be covered but the liquid will not overflow the container.

By this method the basal plate and pedicel of each scale are lightly but distinctly stained while the spine remains unstained. Apparently the so-called dentine takes the stain in much the same manner as has long been known to be the case with bone[3] and the true dentine of teeth. This material is very satisfactory for the study of scale types and their distribution. It is, however, far from ideal for photomicrography. The transparency of the spines necessitates a very careful selection of the areas to be photographed and patient experimentation with light filters and illumination.

[3]Dawson, Alden B. 1926. A note on the staining of the skeleton of cleared specimens with alizarin red S. Stain Techn., 1, 123–4.

THE USE OF AGAR AS A MATRIX FOR SECTIONING PLANT MATERIAL WITH THE FREEZING MICROTOME

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In the course of histological research and continued use of the freezing microtome it has become apparent to the writers that the use of water as a freezing matrix for plant material is generally unsatisfactory. The fact that water is in a hard brittle condition in the solid state, with no controllable gradation of hardness, renders its use difficult and often impossible. The use of a 5% solution of

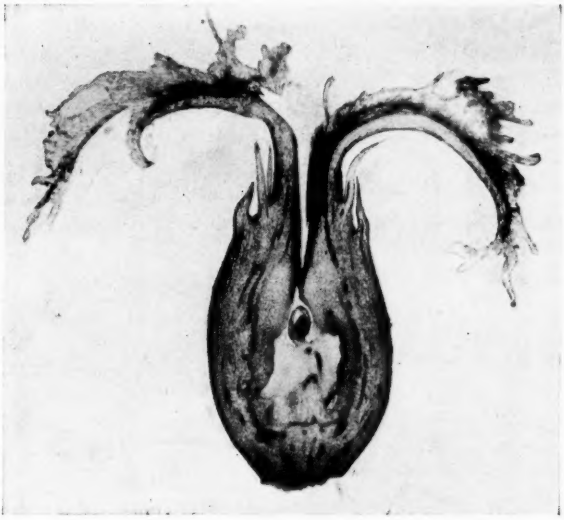

FIG. 1. Use of agar as a freezing matrix. Walnut flower. (Note condition of delicate stigmas.) Section 40μ—magnification approx. 50×.

agar in water has been found by the writers to be of decided advantage. From the seeming lack of references in the literature to the use of agar for this purpose, the writers deem an explanation of their method to be of value.

METHOD

Material is taken from the fixative and rinsed in water (complete washing is more quickly done after the sections are made). The material is trimmed as desired and immersed in the 5% agar which is then allowed to cool. For small material, Petri dishes make excellent

forms and containers for the agar blocks. Larger material may be embedded in paper trays such as are used for paraffin embedding.

Practice is needed in judging the hardness of the material as the sections are cut. The operator should attempt before each cut is made to partially thaw the material to a depth approximating the thickness of the section desired. To aid in this the tip of the finger may be rubbed gently over the material surface or a camel hair brush moistened with 50% alcohol may be applied. Alcohol stronger than 50% should not be used as it too greatly retards freezing.

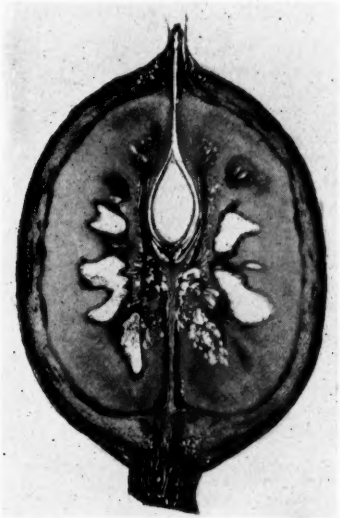

FIG. 2. Use of agar as a freezing matrix. Immature walnut. (Cavities of large material, previously cut in slabs, are filled and supported by agar.) Section 40μ—magnification approx. 4×.

The qualities of the agar gel desirable for this use appear to decrease with age or remelting. For the best results it has been found advisable not to remelt the stock agar gel more than 3 or 4 times. Agar blocks which are not to be sectioned immediately should be wrapped in wet paper toweling and arranged with a wick and water reservoir to insure a constant moisture supply—the whole being covered with a bell jar. Agar blocks so protected from water loss have been kept for 2 or 3 weeks with no apparent harm to gross morphological detail.

Note: The only reference to such use of agar which the writers have at present is a brief note in "Manual on Use of the Microtome," Ernst Leitz, Wetzlar, with the citation to "Zeitschrift für wissenschaftliche Mikroskopie und für Mikroskopische technik," **23,** 328. 1906.

STAINING PARAFFIN SECTIONS WITH PROTARGOL

1. EXPERIMENTS WITH BODIAN'S METHOD. 2. USE OF *N*-PROPYL AND *N*-BUTYL ALCOHOL IN HOFKER'S FIXATIVE[1]

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Abstract.—**Paraffin sections of nervous tissue, which had been fixed in Hofker's fluid, stained readily with protargol solution without the addition of metallic copper or other activator. Amidol-sulfite mixtures reduced the protargol more rapidly and completely than hydroquinone-sulfite. Intensification of the stain could be secured by reducing with 0.5% amidol (or pyrogallol) solution after gold toning. The completeness of staining of unmyelinated fibers of the dorsal roots of cat spinal nerves was checked by estimating the number of fibers in a root and the cells of its associated ganglion. A fiber cell ratio of 1:1 was found in 4 specimens, indicating within limits of error that all fibers were stained. An improvement of the original Hofker's mixture as a fixative was obtained by using a mixture of formic acid, 5 cc.; trichloracetic acid, 10 g.; *n*-propyl alcohol, 20 cc.; and *n*-butyl alcohol, 60 cc. (instead of the acetic, trichloracetic, ethyl alcohol mixture used in the original formula). The following arbitrary method is suggested. Fix 12 to 24 hours, pass to water thru graded ethyl alcohol, wash several hours, dehydrate and embed in paraffin. Cut, mount, and remove the paraffin, pass to water and impregnate 2 or 3 days at 27 to 30°C. in a 0.5% aqueous solution of protargol (Winthrop Chemical Co.). Rinse 2 or 3 seconds and reduce with 0.5% amidol (Agfa brand used) in 5% sodium sulfite solution. Wash, tone with 0.1% gold chloride, wash and reduce with 0.5% amidol (no sulfite), wash, dehydrate and cover. The method works well on spinal nerve roots, cerebrum, cerebellum, and spinal cord, and moderately well on nerve trunks including sympathetic nerves. Tissues from cat and guinea pig were used.**

Part 1. Experiments with Bodian's Method

The introduction of a method based on the use of protargol[2] as the staining agent by Bodian (1936) has made available a neurolog-

[1]Contribution No. 270.

[2]Winthrop Chemical Co., New York, N. Y.

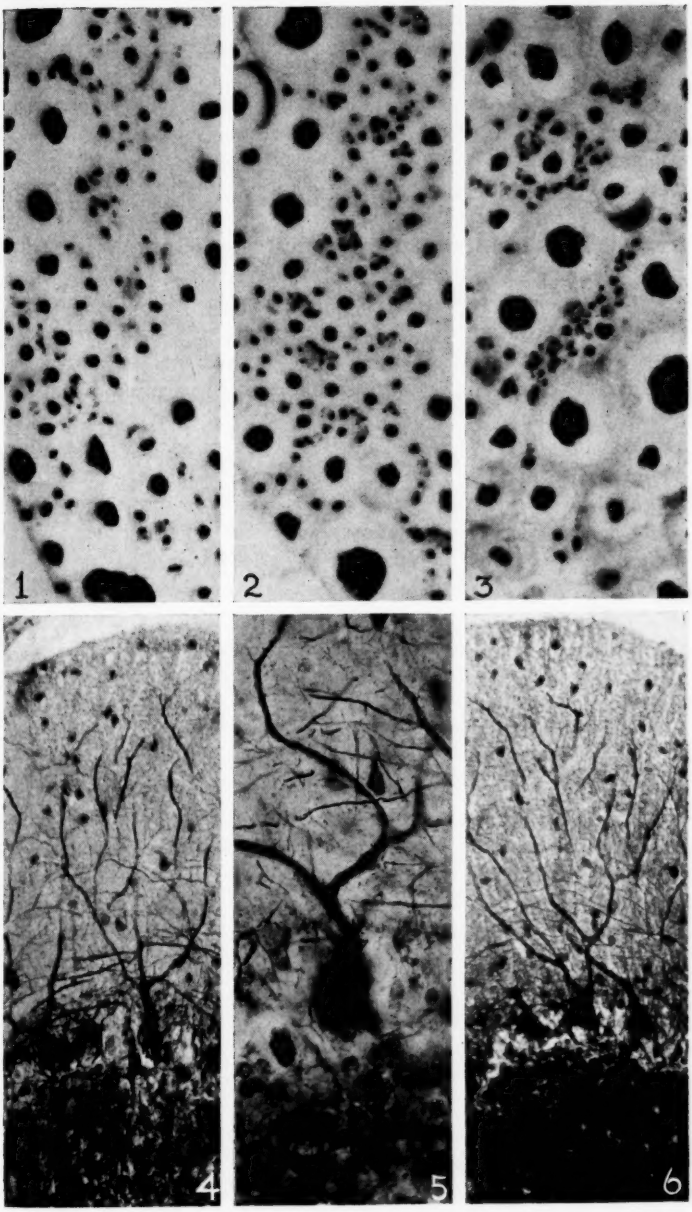
PLATE I, EXPLANATION OF FIGURES

All preparations were stained 3 days in 0.5% aq. protargol solution without copper. Fixative: formic 5%; trichloracetic, 10% in different alcohols. All photographs are unretouched.

Figs. 1 and 2 are from different slides of a 3rd sacral root of cat fixed in the acid mixture in ethyl alcohol, and are representative areas from Rootlet 2, Table 1. Reduction after gold toning (Fig. 2) shows a 45% increase of small fibers over the number shown in the preparation of Figure 1, not reduced after toning. ×1400.

Fig. 3. Area from a 4th thoracic dorsal root fixed in the acid mixture in *n*-propyl alcohol; (Left 4th thoracic) of Table 2. Amidol-sulfite sulfite reduction after silver impregnation and reduced with dilute amidol after gold toning. This shows practically complete staining and resolution of small fibers. ×1100.

Figs. 4, 5 and 6 are areas from the same slide of several sections of cat cerebellum. The sections of one end of the slide (4 and 5) were not reduced after gold toning while those on the other end were (6); 4 and 5 show baskets and climbing fibers best while the tangential fibers are not well stained; 6 has the Purkinje cell dendrites too deeply stained to see the climbing fibers readily but the number of tangential fibers are considerably increased. Fixative: acid mixture in propyl-butyl alcohol mixture. The section of Fig. 6 required 3 times as much exposure to photograph as the sections shown in 4 and 5 on account of the greater density of the background resulting from the second reduction. Figs. 4 and 6, ×200. Figure 5, ×400.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ical stain of unusual merit. Freedom from precipitate and a high degree of differentiation of nerve fibers characterize stains made by the method. When we attempted to use it to stain dorsal roots of mammalian nerves, however, the usual difficulty of incomplete staining of unmyelinated fibers was encountered. While experimenting with modifications of the method designed to overcome this shortcoming, some results of general interest were obtained.

Criteria for testing: The completeness of a silver impregnation of nerve fibers can be tested quantitatively as follows. The recent work by Duncan and Keyser (1938) shows that when the dorsal roots of cat spinal nerves are adequately stained there is the same number of fibers in the dorsal root as there are cells in the dorsal root ganglion, hence the potentialities of a staining method can be ascertained by checking the number of fibers it reveals in a dorsal root against the number of cells in the sensory ganglion. Another criterion is the checking of the number of fibers stained in a single rootlet by variations in staining procedures. The latter gives only comparative data but can be carried out easily by preparing a number of slides of the same dorsal root, subjecting them to variations of staining, and counting the fibers in a rootlet which appears thruout the series. Both of these criteria have been used in the present experiments and also qualitative observations on the staining of the sympathetic trunk, spinal cord and cerebellum.

Comment on previous experience with dorsal roots: The present attempt to secure more complete staining in dorsal roots of cat was prompted by a failure to secure more than an occasional good stain by section staining methods used by Barnes and Davenport (1937). At that time the most promising fixative was Hofker's mixture (1921). Sections of material fixed in this fluid were less distorted than those fixed in acid formalin, and when the unmyelinated fibers could be stained, the fiber number tended to be greater than that of the preparations fixed in acid formalin. Sections coated with nitrocellulose and impregnated by alcoholic silver nitrate (method of Davenport, 1930) were used, but the method was never very satisfactory, due apparently to the fact that the silver impregnation best followed acid formalin (an inadequate fixation) and only occasionally followed Hofker's fixative (a superior fixation).

The block method of Ranson (1911), used by Duncan and Keyser and by Barnes and Davenport, has frequently yielded a count of fibers in the dorsal root which equalled the number of cells in the ganglion. Also the potential ability of the method to stain unmyelinated fibers completely has been verified on the vagus nerve of cat by Foley and Du Bois (1937) and by Jones (1937).

General comment on silver staining of nerve fibers: It is well known by users of silver stains that a method worked out to produce the best results on one type of material is not necessarily equally excellent for others. It can be assumed, however, that a type of stain which impregnates the finer unmyelinated fibers in dorsal roots (for example Ranson's method, referred to above) has a better chance of staining this type of fiber elsewhere than a method which stains such fibers in the roots with difficulty or not at all.

The more complete stains of roots or peripheral nerves appear to be neurofibrillar in character. When nerve cells are included, their nuclei are palely stained while the cytoplasm appears dark with the neurofibrillar network (Figs. 11 and 12). The larger myelinated fibers should not be coal black (largest fiber in Fig. 10), but should show differentiation between the neurofibrils and neuroplasm. Incidentally, the connective tissue background should show sufficient staining to permit ready recognition of all the elements in it. A completely unstained background may well give rise to the suspicion that many of the finer nerve fibers have been "differentiated out" of the picture, particularly if the coarse fibers are intensely stained (Fig. 1).

Material and routine technic: Sacral and mid-thoracic spinal nerve roots, cerebellum, spinal cord and sympathetic trunk of cat were used for test material. The specimens were taken from decerebrated animals and also from others anesthetized with ether or pentobarbital-sodium (Nembutal). Dissections were completed within 2.5 hours after death, and drying during dissection was prevented by moistening with physiological salt solution. The nerves were gently stretched on narrow strips of library card (about 6 or 7 mm. wide) with slits cut in each end to hold either the nerve directly if it were long enough, or to hold the thread used in tying the ends of the shorter nerves. Such card strips, when bowed, exert sufficient tension to keep small nerves straight without overstretching them.

All fixation was by immersion, since it was not feasible to use different fixatives on the same animal by perfusion, and the time of fixation was about 18 hours. Specimens were transferred from the fixative to 95% alcohol, then 80%, 50% and finally washed in water for 4 to 6 hours. The washing was followed by alcohols of ascending concentration to absolute then thru two changes of normal butyl alcohol as recommended by Hewitt (1931) and embedded in paraffin.

Most of the earlier staining experiments were made with material fixed in unmodified Hofker's fluid.[3] The modifications of the fixative will be described in Part 2 of this paper. Modifications of the fixative

[3]Trichloracetic acid, 1 part; glacial acetic acid, 1 part; abs. ethyl alcohol, 8 parts.

PLATE 2, EXPLANATION OF FIGURES

Fixation and staining were the same as that of the preparations shown in Plate 1 except the dorsal root ganglion cells, Fig. 11. This preparation was given a preliminary impregnation of 1 day in 3% aq. silver nitrate and then transferred to 1.5% protargol for 2 days.

Figs. 7 and 8 illustrate the greater tendency toward cracking of the tissue when ethyl (or methyl) alcohol is used for the solvent of the acids in the fixing mixture. Fig. 7 is an area from the dorsal root of a 5th left thoracic nerve fixed in acid ethyl alcohol; Fig. 8 is taken from the right companion nerve root fixed in acid normal propyl, normal butyl mixture 1:3 parts, respectively. Cracking has been rare after the latter type of fixation. ×260.

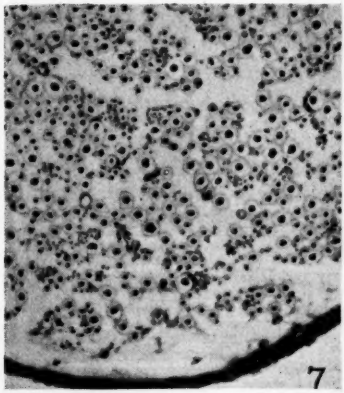
Fig. 9 is a high power (×1900) of a representative area of Fig. 8. The number of fibers in this root was estimated to be 91% of the number of cells in its ganglion (Right fifth thoracic), (Table 2).

Fig. 10. A splanchnic branch of the sympathetic trunk fixed in the acid mixture in methyl alcohol. A wide range of fiber size is seen. ×1200.

Fig. 11. Dorsal root ganglion cell which illustrates the neuro-fibrillar character of the type of stain described in the text. This preparation was reduced after impregnation with Bodian's reducing mixture and treated with oxalic acid after gold toning. (Oxalic acid does not reduce the AgCl formed in the tissue during toning.) ×1000.

Fig. 12. Motor cell from the lumbar enlargement of cat spinal cord. Neurofibrills are prominent in the diagonally cut end of the dendrite in the upper part of the figure. Near the middle, synaptic end-bulbs appear as rings. ×900.

All of the above preparations except Fig. 11 were reduced after gold toning with 0.5 to 1% amidol solution, without sulfite.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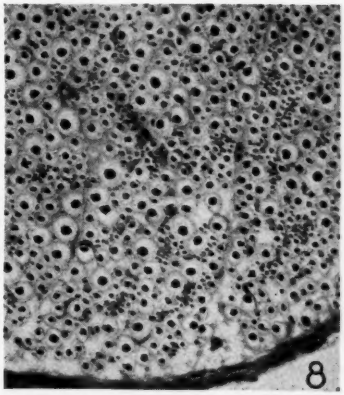
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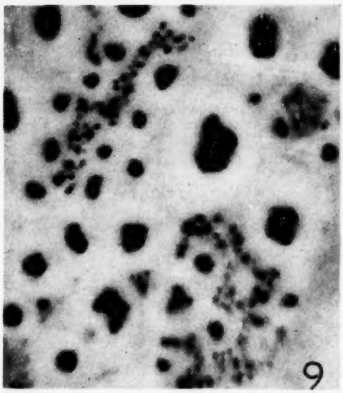
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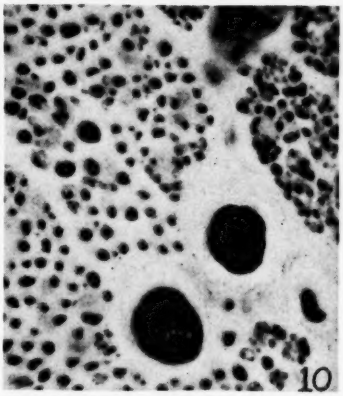
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had no marked effect on the staining; hence later staining experiments with modified fixatives are comparable to, and included with, earlier ones in the following paragraphs.

Sections were cut 6–12μ thick and affixed to slides in the usual manner with albumin fixative.

Staining with silver nitrate: Prior to experiments with protargol, attempts were made to stain bare sections with silver nitrate. The imperfect success of this attempt does not merit recommending it for routine purposes, but it seems of interest that when material, prepared as described above, is impregnated in 2 or 3% aqueous silver nitrate for 2 days at 27 to 30°C. and reduced with 10% pyrogallol in 25% alcohol, a number of specimens show good differentiation of axis cylinders with complete or nearly complete staining of even the finest fibers. In order to minimize the formation of precipitate the pyro solution was dropped onto the slide and allowed to flow across it. It was found that impregnation with more dilute silver nitrate (1% or weaker) still further reduced the amount of precipitate, but in order to bring out the finer fibers it was necessary to reduce a second time after toning with 0.1% gold chloride. This second reduction is an important part of the technic when protargol is used as the impregnating agent and will be discussed under gold toning.

Protargol impregnation and effect of pH: Protargol exhibits an isoelectric point roughly about pH 4, at which acidity it is precipitated, hence it might be expected to behave somewhat like a dye by having its selectivity altered by variation of pH of the staining mixture. A few experiments of this kind indicated that acidification from neutrality to pH 5 or 6 may slightly enhance its affinity for nerve fibers (confirming Bodian) but that alkalinization definitely impairs its action. In the presence of copper, acidification seemed to be without effect.

Protargol silver nitrate combinations: Since silver nitrate solutions had been found to impregnate even the finest fibers, it was assumed that the addition of a small amount of silver nitrate to protargol or the addition of protargol to a silver nitrate solution and filtering off the precipitated protein might improve the staining reaction. A 1.5% protargol solution could be diluted with an equal volume of 0.05% silver nitrate without precipitation, but this mixture used as a staining solution seemed to avoid almost specifically the fine unmyelinated fibers. The addition of 5 cc. of 1% protargol to 50 cc. of 3% silver nitrate solution, and removal of the precipitated protein by filtration, gave a staining solution which stained fine fibers but they were inadequately differentiated from a rather deeply stained

background. Attempts to combine silver nitrate and protargol were abandoned when it was found that progressive diminution of the concentration of silver nitrate added to protargol resulted in progressively better staining.

The effect of adding copper to protargol solutions: The addition of metallic copper according to Bodian's method causes a progressive reduction in the concentration of protargol (by replacing the combined silver) during the staining reaction. Stains were made with copper added to 1% protargol and compared to those made without copper when protargol concentrations of 0.75%, 0.25% or 0.1% were used on material cut from the same block of tissue. The effect of the copper was to decrease the selectivity of the stain without gain or even a loss of staining of finer fibers. It should be noted, however, that the observation that the addition of copper was deleterious applies to Hofker-fixed material. Whether the same is true for formalin fixed tissue has not been tested in the present series of experiments.

The essential effect of the addition of copper was found to be that of accelerating the rate of impregnation. Whereas impregnation could be accomplished in 1 day at 27 to 30°C. when copper was added, 3 days were required with a plain protargol solution. The acceleration effect was noted also when a spent protargol-copper solution was added to the extent of 10% by volume to a fresh 1% protargol solution, but whether copper was added to a fresh solution or a similar fresh solution "activated" with a small amount of used solution, the results were always inferior to those obtained with protargol alone provided sufficient time were allowed for impregnation.

The possibility that omission of the copper from the protargol bath might result in improvement in staining after fixatives other than Hofker's fixative should not be overlooked. Of greater importance, however, than the addition or non-addition of copper seems to be processing subsequent to impregnation.

Concentration of protargol: Solutions of strength varying from 0.1% to 1.5% were tried; 0.1% and 0.25% were too dilute to yield good stains consistently; 0.5% and 0.75% seemed adequate and higher concentrations gave no apparent improvement. The concentration is not very critical and 0.5% has been used as a rule.

Repeated use of the same solution: When no copper is added, the deterioration of the staining solution is rather slow provided it is not accidentally contaminated with the reducing solution. Such contamination spoils the selectivity of the stain. We have used one bath six times over a period of a month but the last stain practically

failed. Since there is always some likelihood of contaiminating the silver bath with reducing solution during the transfer of slides, it would seem to be good routine to use the silver solution only once.

Testing different batches of protargol: Three different factory batches were tried simultaneously and staining appeared to be very uniform.

Reduction of protargol: Seven reducing agents (hydroquinone, bromhydroquinone, pyrogallol, elon, amidol, tannic acid and gallic acid) were tried in combination with sodium sulfite, sulfite-bisulfite mixtures, or sulfite-carbonate mixtures. Of the lot, amidol (Agfa brand used) was easily best for general use. Hydroquinone, bromhydroquinone, or pyrogallol all effected an apparently good reduction when used as 0.5 to 1.0% solutions in 5 to 10% sodium sulfite and 2% sodium carbonate. Tannic and gallic acids were too sluggish even in the alkalinized solutions and tended to give a coarse deposit of reduced silver. Elon generally failed to give adequate differentiation of nerve fibers, altho in combination with hydroquinone as used in a carbonate-sulfite photographic developer the reduction appeared to be quite satisfactory. Amidol was found to function in sulfite-bisulfite mixtures at pH 6 whereas none of the other reducing agents were active at this pH. It was somewhat too active in sulfite-carbonate mixtures (pH about 10) hence should be used with sulfite alone, or with sulfite-bisulfite mixtures.

Hydroquinone and amidol were tried alone in 1% solutions without sulfite and the function of the sulfite as a differentiating agent was clearly shown by the experiment. Preparations so reduced showed very little differentiation of nerve fibers, for the connective tissue stained with an intensity equal to the nerve fibers. Sulfite alone caused some reduction in nerve fibers but seemed to remove the protargol from the tissue rather rapidly as preparations treated first with sulfite solution could not be subsequently reduced with amidol-sulfite solution.

The interaction of sulfite and the more reactive reducing agent furnishes the means of some control over the degree of differentiation of nervous elements, for when the sulfite concentration was high (10%) and that of amidol low (0.25%) a greater degree of differentiation was obtained than with 2.5% sulfite plus 1% amidol.

The effect of reduction of protargol under different conditions and the subsequent reduction of silver chloride deposited during gold toning will be shown in the section on numerical data.

Rinsing slides before reduction: Rinsing is unnecessary but a few seconds rinse in tap water or distilled water was used in numerous

trials without evident impairment of the stain. It was found, however, that rinsing in dilute acetic acid removed the protargol from the tissue, but that rinsing in dilute sodium carbonate did not. The dilute acetic acid (pH about 3.5) was on the acid side of the isoelectric point of protargol and would, therefore, seem to have removed it from the tissue by the mechanism of salt formation in a manner similar to that of acid differentiators acting on basic dye stains.

Time of reduction: When amidol was used the reduction was practically complete in 1 minute. Other reducing agents reacted somewhat more slowly. The pH of the reducing solution affected the time required for complete reduction; in general, the more alkaline the solution the faster the reduction.

The second reduction: The intensity of the stain after the reduction of the protargol may or may not be sufficiently dark. If it is too light, a second reduction after gold toning has been found to be a very effective means of increasing intensity. When this procedure was used it was found that the concentration of the gold chloride used in toning was uncritical except that the more concentrated solutions toned faster than dilute ones; 0.1% was ordinarily used. The customary addition of acetic acid to the toning solution was omitted as it appeared to be superfluous. When the reaction $3Ag+AuCl_3=3AgCl+Au$ was completed, as shown by the graying of the sections, the excess gold chloride was removed by washing 1 or 2 minutes in running water and the silver chloride, which remained in the tissue, was reduced by dropping a 0.5 to 1% amidol solution (without sulfite) on the slide. Reduction occurred in a few seconds. Treatment with a solution of sodium thiosulfate effected little or no change in the appearance of the stained material after such reduction since the silver chloride had been reduced to metallic silver.

The second reduction was found very advantageous to enhance the visibility of the very fine unmyelinated fibers of the dorsal root (compare Figs. 1 and 2). If the reduction of the protargol (first reduction) was sufficiently vigorous, most of the fibers were stained without a second reduction as shown by preparation 3, Table 1.

Data on fiber counts: Table 1 gives the number of fibers found in two specimens of sacral rootlets in which the main variations were in the pH of the reducing solution and whether or not a second reduction followed gold toning. The preparations not reduced after toning were treated with oxalic acid as recommended by Bodian.

The counts of the fibers stained in the above preparations show that when hydroquinone-sulfite was not followed by reduction after gold toning (i.e., oxalic acid and hypo) there was considerable likelihood

that many fine fibers remained invisible. The presence of copper in the protargol staining solution did not alter the final results (416 and 421 fibers in slides 1 and 2 of rootlet 1). The use of a more vigorous reduction of the protargol by a reducing solution containing carbonate increased the number to 874 (slide 3). The additional reduction with pyro solution after gold toning further increased the number of fibers even after hydroquinone-sulfite reduction of the protargol.

Rootlet 2 shows similar results in that there is an increase of stained fibers from 489 to 708 as a result of reducing after toning. In these preparations a moderately active first reducer was used (Figs. 1 and 2).

TABLE 1. THE NUMBER OF NERVE FIBERS STAINED BY DIFFERENT METHODS IN 2 ROOTLETS OF SACRAL DORSAL ROOTS OF CAT.

Slide No.	Staining solution	Protargol reduced by	Reduction after toning	Number of fibers
		ROOTLET 1.		
1	¾% Pr.	Hq-sulf.	None	416
2	1% Pr.+Cu	Hq-sulf.	None	421
3	¾% Pr.	Photo.	None	874
4	¾% Pr.	Hq-sulf.	Pyrogallol	935
5	¾% Pr.	Photo.	Pyrogallol	923
		ROOTLET 2.		
1	½% Pr.	Elon-sulf-carb.	None	489
2	½% Pr.	Elon-sulf-carb.	Pyrogallol	708

Abbreviations:
Pr.—protargol
Hq-sulf.—1% hydroquinone in 5% Na_2So_3
Photo.—photographic developer (elon, 0.1%; Hq, 0.8%; Na_2So_3, 2%; Na_2CO_3, 2%)
Elon-sulf-carb.—elon, 1%; sulfite, 5%; carbonate, 2%.

Amidol was not tried under similar conditions but was used in the first and second reductions of other dorsal root preparations of thoracic nerves. The fiber numbers in four such roots were estimated and the cells in the corresponding ganglion were estimated also. Table 2 shows the numbers obtained.

We believe that the close agreement between fiber number and cell number is the best available evidence that the modifications applied to the Bodian method were effective in staining all the fibers in cat dorsal roots. We have attempted to give the basis for the modifications and to subject some of the stained preparations to a critical test, the enumeration of fibers. Probably no silver method stains all of the fibers all of the time, but the method given in the abstract of this paper has given the most consistent results.

Part 2. Use of n-Propyl and n-Butyl Alcohol in Hofker's Fixative

In an attempt to improve the fixation of spinal roots and peripheral nerves, various fluids were used as substitutes for ethyl alcohol in Hofker's mixture. Methyl, *n*-propyl, *n*-butyl, and tertiary butyl alcohols, acetone, and a mixture of 25% *n*-propyl to 75% *n*-butyl were used as the solvents for the trichloracetic-acetic mixture originally recommended by Hofker. At first it was supposed erroneously that methyl alcohol would be superior to ethyl because its smaller molecule would facilitate more rapid penetration. The results, however, were in opposition to this as judged by the amount of distortion

Table 2. Number of Fibers in Dorsal Roots and Cells in the Associated Ganglion of Thoracic Nerves of Cat.

Side and spinal segment	No. fibers in root	No. cells in gang.
Right 4th thoracic	7,000	6,860
Left 4th thoracic*	7,800	7,900
Right 5th thoracic*	8,000	8,800
Right 6th thoracic	8,000	7,600

*Sample areas of the cross sections of these roots are shown in Figs. 3, 7 and 8.

and shrinkage as well as by the type of staining of fine fibers obtained by protargol. The effect of the various solvents on fixation can be summarized as follows:

1. Methyl alcohol: Myelinated fibers were distorted and often widely separated; unmyelinated fibers stained poorly and sometimes formed clumps. One good preparation of sympathetic trunk of cat was, however, obtained.
2. Ethyl alcohol: Frequently failed to give good fixation but was generally better than methyl. Preparations usually stained well.
3. *N*-propyl alcohol: Was superior to ethyl in dorsal roots and sciatic nerves; about the same as ethyl for sympathetic trunk. Staining reactions were excellent.
4. *N*-butyl alcohol: Preparations showed slightly more shrinkage than those obtained from propyl. Myelin sheaths were slightly less round.
5. Acetone[4]: Similar to ethyl alcohol.
6. Tertiary butyl alcohol: Similar to methyl alcohol.
7. Propyl-butyl mixture: As good or slightly better than propyl alone.

The conclusion is that either normal propyl alcohol, or possibly better still a mixture of 25% propyl in butyl alcohol, is a des rable

[4]Possesses a theoretical advantage in that it does not esterify with the acids used in the fixing mixture.

modification of the solvent used for the acids in Hofker's fixative when it is used for nerves. A successful arbitrary formula for such fixative was:

Trichloracetic acid	10 g.
Glacial acetic acid	10 cc.
Normal butyl alcohol	60 cc.
Normal propyl alcohol	20 cc.

The alcohol mixture was added to the acids immediately before use because they esterified on aging. Another formula which gave equally good fixation and possibly better staining was:

Trichloracetic acid	10 g.
Pure formic acid	5 cc.
Normal butyl alcohol	60 cc.
Normal propyl alcohol	20 cc.

The object in the addition of the propyl alcohol to butyl was to make the mixture a better absorber of the water derived from the tissue during fixation. Since the results obtained with propyl alone were essentially equal to those obtained by the mixture the proportion of propyl added is likely to be rather uncritical.

The above mixtures have been tried on cerebellum and spinal cord of cat with results that seem definitely better than similar acid mixtures made up with ethyl or methyl alcohol. Roots and sciatic nerve from guinea pig gave results similar to those from cat.

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PERMANENT PREPARATIONS FROM RAPID CYTOLOGICAL TECHNICS

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ABSTRACT.—**The following rapid but reliable method of making permanent preparations from temporary mounts has proved to be very useful.**

Pollen mother-cell smears: Smeared anthers are treated in the usual way with Belling's aceto-carmine, except that the cover slip is left off. When correct differentiation is attained the stain is thoroly washed off with 50% acetic acid and the slide flooded with dioxan. This is followed by 2 changes of dioxan for 2 minutes each. A drop of Canada balsam dissolved in dioxan is added and a cover slip applied. In cases where a cover slip has been used at the aceto-carmine stage it can be floated off in a staining jar of 50% acetic acid and dehydration with dioxan carried out as above.

Insect salivary gland chromosome smears: The glands are crushed under a cover slip in aceto-carmine on a slide coated with dried egg albumen. After 20 minutes the area around the cover slip is flooded with 50% acetic acid and the cover slip floats loose so that it can be removed. The above described dioxan dehydrating procedure is then employed.

Squash preparations: Root tips are fixed in some suitable fixative and the Feulgen technic applied. The stained root tips can either be dehydrated by passing thru 3 changes of dioxan and mounting in dioxan-balsam where they are divided into small longitudinal sections by sharp needles, or they can be put immediately into a mixture of 1 part of 50% acetic acid to 1 part of corn syrup where shredding with needles is carried out. A cover slip is put on and separation of the cells completed by tamping or by applying pressure to the cover. This squash method is useful with anthers which are difficult to smear when in the early prophase stages of meiosis.

In recent years rapid methods of cytological technic have become increasingly important and widely used. These methods are, for the most part, simple and rapid in their application and produce excellent results, but there is no equally simple method of making these preparations permanent which will preserve them so that they can

[1]The writer wishes to express his gratitude to Dr. L. C. Coleman for his kind help and valuable suggestions.

be set aside and examined at leisure. This paper presents methods which have been successfully used for some time to make permanent preparations from temporary mounts without shrinkage, distortion, or loss of brilliance of the stain.

Permanent Aceto-carmine Smear Preparations

Pollen mother-cell smears: Anther smears are treated in the usual way with Belling's iron-aceto-carmine, except that it is better to leave the cover slip off if it is known that the mount is to be made permanent. When proper differentiation between chromosomes and cytoplasm has been attained, the stain is removed and the slide gently washed with 50% acetic acid until excess stain is removed. The acid is left on the slide for about a minute after the washing. It is important to remove all excess stain and to have the acid colorless after being used for washing, because dioxan tends to precipitate the excess stain, causing a darkening of the cytoplasm. Dioxan is then added to the acid on the slide and the mixture drained off. The slide is flooded with dioxan for 2 minutes. This dioxan is removed and replaced with fresh, which is drained off after 2 minutes; then a drop of Canada balsam dissolved in dioxan is added, and a cover slip put on. It has been found in some cases, if, instead of following the final change of dioxan with dioxan-balsam, xylene is applied so that it drives off the remaining dioxan, and if then the slide is stood in a staining jar of xylene for 5 to 10 minutes and mounted in xylene-balsam, a slightly sharper and clearer preparation is obtained.

In the case of semi-permanent preparations that have been ringed or sealed for some time and have lost the contrast between chromosomes and cytoplasm, the ringing compound can be loosened and the cover slip floated off in a staining jar of 50% acetic acid. The slide can then be examined under the microscope and destained in 50% acetic acid until correct differentiation between chromosomes and cytoplasm is obtained. Sometimes it is impossible to obtain proper contrast by destaining, or the destaining goes too far while the cover slip is being removed; in such cases, it is better to destain completely and restain with aceto-carmine. This gives results equal to the original and the preparations can be made permanent by the above-described dioxan dehydrating procedure.

Pollen grain smears: This technic can be applied to pollen grains at the time of the first mitosis. Here it is advisable to mount in immersion cedar oil instead of balsam to prevent the collapse of the pollen grain walls which usually occurs in balsam even if it is very dilute. This requires a slight modification of the technic: the last

The photomicrographs are of permanent preparations, all dehydrated with dioxan and mounted in dioxan-balsam.

Fig. 1. Pollen mother-cell smear of *Tradescantia paludosa* stained with aceto-carmine. (Pre-treated with sodium acetate 2^{-6}mol. solution for 15 sec. to loosen the chromonematic coils.) ×1000.

Fig. 2. Salivary gland chromosomes of *Drosophila melanogaster* stained with aceto-carmine. ×750.

Fig. 3. Chromosomes of *Vicia faba* from a root tip squash preparation fixed in Belling's modification of Navaschin's fixative and stained by the Feulgen technic. ×1000.

Fig. 4. Nucleus of *Lilium erabu* showing the pachytene stage of meiosis: From an anther squash preparation fixed in Belling's fixative and stained with the Feulgen nuclear stain. ×750.

change of dioxan is followed by a mixture of 1 part of dioxan to 2 parts of cedar wood clearing oil for 3 minutes, then 1 part of dioxan to 2 parts of oil for 3 minutes. This is drained off, the excess blotted away, a drop of immersion oil added and a cover slip put on. While this technic for pollen grains is reasonably rapid, the results are decidedly inferior to those obtained when the Feulgen technic is used, which is well worth the extra time and trouble.

Insect salivary gland smears: *Drosophila* salivary gland chromosome preparations stained with aceto-carmine can be made permanent by the dioxan procedure without shrinkage or change occurring. In this case, the cover slip is put on and the glands crushed in the aceto-carmine. The preparation is left in this state for about 20 minutes and then the area around the cover slip is flooded with 50% acetic acid. After a few minutes the cover slip floats loose and can be lifted off. If the slide has been previously smeared with a thin film of egg albumen as suggested by Bauer (1936), most of the material remains on the slide. If it is not coated with dried egg albumen most of the material clings to the cover slip and it as well as the slide must be treated by the dioxan dehydrating process.

Smears of insect spermatocytes can be made permanent in the same way, but like pollen grains they are not as clear as when fixed in some suitable fixative and the Feulgen nuclear stain used.

Mossman (1937) in his comprehensive paper on the dioxan technic states: "From the Department of Embryology of the Carnegie Institute at Baltimore, reports have come of excellent results with this reagent in the dehydration of insect salivary gland chromosome smears stained in aceto-carmine and of insect spermatocyte preparations fixed in Gilson's fluid and stained in iron hematoxylin." No details of the process are given. Bridges (1937) suggests the use of dioxan in the vapor method of changing reagents, which seems unnecessarily complicated as compared to the liquid method.

Permanent Squash Preparations

Root tip squash preparations: The "Nuclealquetschmethode" of Heitz (1936) gives an excellent rapid means for making chromosome counts and studying the finer details of chromosome morphology in somatic tissue. The cells remain whole and can be well flattened so that the entire chromosome complement lies in one plane. With this method the Feulgen technic is used. (See Feulgen and Rossenbeck, 1924.) Root tips are fixed in chromosmic or some other suitable fixative for from 30 to 60 minutes, washed thoroly and placed in normal HCl at 60°C. for 5 to 15 minutes depending on the fixative

used. This treatment, besides converting the thymonucleic acid into an aldehyde which reacts with the leuco basic fuchsin, dissolves the middle lamellae of the cell walls and brings about a separation of the cells. After this acid treatment, the root tips are rapidly washed and transferred to the leuco basic fuchsin[2] for 20 to 30 minutes or longer. They are then put thru 3 changes of sulphur dioxide water of 10 minutes each. Two methods of making good permanent mounts of these root tips have proved satisfactory.

The first involves the use of dioxan. The root tips are dehydrated in 3 changes of dioxan of 3 minutes each and a single root tip is placed in a drop of dioxan-balsam on a slide where it is carefully divided into small narrow longitudinal strips by means of very sharp needles. A cover slip is put on and the separation of the cells completed by tamping the cover with the blunt end of a pencil or needle holder. With some roots that do not shred easily with needles, smearing on a slide with a scalpel before the dioxan treatment is necessary, but care must be taken not to put too much pressure on the scalpel or the cells will be mashed and distorted. Another alternative with which good results have been obtained is by a method similar to that used with the salivary glands of *Drosophila*. The root tips are partially shredded by needles and crushed under a cover slip in 50% acetic acid on a slide coated with dried egg albumen and the dioxan procedure that was followed for the glands is used.

The second method of making permanent preparations of these root tips is much simpler and quicker than the first. Here, corn syrup is used as the mounting medium. After staining and bleaching, the root tips are transferred to a mixture of 1 part of 50% acetic acid to 1 part of corn syrup. Separation with needles is carried out at this stage, a cover slip is applied, and spreading of the cells completed by tamping. Mounts made by this method have good optical properties which have been retained after a year and a half. Other advantages of this method are speed, economy, simplicity, and the fact that the cells tend to separate and spread more easily in the acetic acid syrup mixture than in balsam. Sometimes the clearing action of dioxan is necessary, however, and in humid weather the corn syrup tends to run. It is usually only the hard layer around the edge of the cover slip that is affected and this can be avoided by ringing.

Root tip macerations mounted in corn syrup are very valuable for large classes. Frequently students get the idea from sectioned material that chromosomes are merely drawings in the frame of the

[2]An improved leuco basic fuchsin which produces superior results is described by Coleman, L. C. Preparation of leuco basic fuchsin for use in the Feulgen reaction Stain Techn., **13**, 123–4. 1938.

cell wall, but with maceration mounts which they can make themselves, they see cells rolling over and over, and get the conception of the cell as a three dimensional unit and the nucleus as something with volume and shape. Where material stained by the Feulgen technic is to be used it is advisable to counterstain with fast green or light green as it tones down the unpleasant magenta color of the chromosomes. If color filters are available, it is not necessary to counterstain, as a combination of green and orange color filters gives a pleasant color and increases the definition tremendously.

Besides the "Nuklealquetschmethode" for root tips, Heitz outlines a "Kochmethode" in which aceto-carmine is used as the stain, without previous fixation. Results with this method, especially for finer morphological details are inferior to those obtained with the Feulgen nuclear stain. Warmke (1935) also employed aceto-carmine, but with previously fixed material in which the cells had been separated by HCl. Good results can be obtained by this method, but the preparations lack the precise staining shown after the Feulgen technic. Permanent preparations can be made from these aceto-carmine technics by the dioxan procedure, but not by the corn syrup method as the staining contrast in this case is not permanent.

Anther squash preparations: Difficulty is often experienced in smearing the anthers of certain plants which are in the leptotene and pachytene stages of the prophase of the first division of meiosis. This is due to the fact that the cells are still in a tissue formation which tends to prevent them from spreading out and separating. Also when in this condition they fail to adhere properly to the slide. With such material the anthers are cut into small pieces of from 1 to 2 mm. in length, fixed in some suitable fixative and stained by the Feulgen technic. The anther segments are then placed in dioxan and their contents poked out with a needle. The pollen mother-cells come out in the form of a sausage-shaped mass, or as smaller masses of cells, which are dehydrated in 2 changes of dioxan for 2 minutes each, mounted in dioxan-balsam and squashed under a cover slip. This gives preparations in which the cells are well flattened and in which the chromatin threads of the nucleus are not stretched or pulled out of position.

The methods for making permanent preparations by the rapid cytological technics described in this paper have been used on a wide range of plant materials including fungi, and with some animal tissue. A few representative examples of these are shown in the photomicrographs. The standard technics do not apply equally well to all types of material, but with slight modifications they are usually

practical. The methods and modifications of these suggested give an idea of the possibilities of such procedures.

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Since writing this paper the squash method has been applied to gynospore (megaspore) and embryo sac development. This ovule squash method, which is essentially similar to the anther squash technic, has proved to be very successful with *Lilium* material. An account of the technic will be published when it has been applied and tested on other types of material.

LABORATORY HINTS

FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

The abstracts given here are intended primarily for laboratory use; consequently the technic in each instance is given in as much detail as possible.

J. A. de Tomasi — *Abstract Editor*

MICROSCOPE AND OTHER APPARATUS

BERNARD, J. E. **The principles of fluorescence microscopy.** *J. Royal Micro. Soc.*, **57**, 256–9. 1937.

This paper is a review of the fundamentals of fluorescence microscopy, supplemented by a brief discussion of the equipment required for medium-power work. With reference to illumination, the arc should be as long and as steady as possible regardless of the type of lamp used. If the cost is not a deciding factor, a quartz optical system is preferred. Of the sensitizers, primulin has so far given best results.—*J. A. de Tomasi.*

WRIGHTON, H. **A new light source for microscopy.** *J. Royal Micro. Soc.*, **57**, 260–1. 1938.

Up to the present the mercury-vapor lamps used in microscopy have been of the low-pressure type. A new product of the General Electric Co. is the "Osira" lamp consisting of a small quartz emission tube ¼ in. across and 1 in. long. This tube is mounted, like any ordinary electric bulb, inside a frosted glass jacket. This lamp can be used for microscopical work when the jacket is removed. It offers the advantage of lighting as soon as the switch is turned on, first with a bluish light, then after a few seconds with a white and very intense emission. This radiation source differs from all others because certain wave-lengths, particularly in the blue band, are much stronger than others. By appropriate separation, strong emission in the ultra-violet as well as monochromatic green or yellow light can easily be obtained.—*J. A. de Tomasi.*

MICROTECHNIC IN GENERAL

COMANDON, J. and DE FONBRUNE, P. **La chambre à huile. Ses avantages pour l'étude des microorganisms vivants, la culture des tissus et la micromanipulation.** *Ann. Inst. Pasteur*, **60**, 113–41. 1938.

For prolonged critical work at high magnifications with living material, a paraffin oil immobilization technic combines simplicity with versatility. A water droplet with the material is injected into a paraffin drop on a cover slip so that the water droplet, adhering to the glass, is surrounded by the oil. The cover slip, held in a special metal clamp, is inverted for observation. Living objects survive indefinitely because the oil is non-toxic and cuts down evaporation, yet permits the ready passage of O_2 and CO_2. Additional water or reagents may be added as desired. The thinness of the aqueous layer permits the use of high N. A. condensers for photomicrography, and displacement of the object is minimized by lack of evaporation-induced diffusion currents. The object may be further restrained if necessary by reducing the volume of water. Objects may be fixed in place with reagents penetrating the oil layer, such as the vapor of osmic acid or formaldehyde. The oil and water are then drained off and the object stained as an ordinary section.

A viewing chamber for securing the thinnest films may be constructed of a large square cover slip supported on a slide by a portion of thin slide on each end of the first slide. The cover slip with the aqueous droplet is first inverted on its supports so that the droplet is flattened by the supporting slide, then the space in the cham-

ber around the water film is filled with paraffin oil, which adheres by capillarity. More elaborate apparatus is described and illustrated for tissue culture, micromanipulation and photomicrography.—*S. H. Hutner.*

ORTON, J. H. **Note on shrinkage of oyster tissues in Bouin's fixative.** *J. Royal Micro. Soc.*, **57**, 255. 1937.

The approximate shrinkage in samples of oyster tissue can be estimated by weighing the specimens fresh, and again after a 1½ month fixation in Bouin's solution. Shrinking appears roughly to run parallel with loss of weight, which averages 8.7% with this particular tissue. Comparing these results with similar work on *Paramecium* indicates that comparable tissues lose about 1/12 of their volume when preserved in Bouin's. As the knowledge of these changes may be a matter of importance in certain specific cases, the suggestion is made that isotonic solutions be investigated with the object of preserving without shrinkage.—*J. A. de Tomasi.*

RICHARDS, O. W. and SMITH, J. A. **Lucite not a substitute for Canada balsam when mounting microscope slides.** *Science*, **87**, 374. 1938.

Lucite, a clear and colorless du Pont plastic is soluble in dioxan and has, therefore, been suggested as a mounting medium in microscopical technic (Science, **85**, 365. 1937). More recent experience shows that such solutions tend to bleach aq. solns. of the following dyes in increasing degree: basic fuchsin, methylene blue, eosin. Heidenhain's hematoxylin, Ehrlich's hematoxylin, acid fuchsin and light green, Other solvents which did not prevent fading were: acetone, amyl acetate, ethyl acetate. Decolorization of stained sections takes place in from a few days to 5 months. Clearing is also less effective than in balsam or dammar. Poor results are obtained in mounts under cover glass.—*J. A. de Tomasi.*

SELBACH, H. **Über Volumänderungen des Gehirngewebes in Pufferlösungen verschiedener pH.** *Zts. gesam. Neurol. u. Psychiatrie*, **162**, 145–59. 1938.

A series of 500 pieces of rabbit brains (250 mg. in each piece) were studied for rate of swelling in a series of Sorensen's phosphate buffer mixtures (pH 6.0-8.3). The least swelling occurred at about pH 7.4. Swelling was more rapid on the alkaline side of the physiological range, a maximum being reached in 2–3 hr., while on the acid side a similar maximum was reached in 6 hr. Possible relationships between equilibration of neural and glial components were discussed; also the similarity between the swelling produced experimentally and that caused by disease.—*H. A. Davenport.*

ANIMAL MICROTECHNIC

BROWNING, P. **A staining method for bone marrow films: wet fixation with Leishman's fluid and staining in a solution of electrically controlled pH.** *J. Path. & Bact.*, **46**, 200–3. 1938.

The bone marrow smear is fixed 1½ min. with Leishman's fluid. Excess is then poured off. The slide, film side down, is placed in Leishman's stain and dist. water 2:1, contained in a depression not more than 1/16 in. in depth molded in paraffin to receive the slide. Direct current is passed thru the bath by means of sheet aluminum electrodes fitted to each end of the slide. Precise control of the neutrality of the stain is managed by noting the migration of the different color zones. The contents of the staining bath are mixed between each staining to maintain the neutral reaction. After staining, the slides are wiped dry around the edges, dehydrated and differentiated in 1.5% colophonium in abs. alcohol, then xylene and balsam. *S. H. Hutner.*

LILLIE, R. D. and PASTERNACK, J. G. **Romanowsky staining with buffered solutions: II. Current modification.** *J. Techn. Methods and Bull. Inst. Assoc. Med. Museums*, **15**, 65–70. 1936.

The polychrome stain in this technic is obtained by prolonged treatment of the methylene blue solution with a Ag salt. It is made into an eosinate and used on material with suitable buffers. Required solns. are prepared as follows:

Stain solution: Dissolve 10 g. $AgNO_3$ in 500 cc. dist. water; add 50–75 cc. of 5% NaOH; wash 5 times by decantation. Add moist precipitate to 2 liters of 1% certified methylene blue. Mix, stopper and shake at intervals for 11 days.

Filter and add to filtrate 1,800 cc. of 1% aq. eosin. Stand overnight, collect and dry ppt. on hard filter paper. Yield should be 35 g. of polychrome blue eosinate. Dissolve into stock soln. (dry stain, 0.6 g.; anhydr. glycerin, 25 cc.; neutral acetone-free methyl alcohol, 85 cc.; stored in small bottles, completely filled).

Citric buffer solution: Dissolve 21.008 g. citric acid, discarding imperfect crystals, in 500 cc. dist. water; add 500 cc. methyl alcohol; mix, cool and make up to 1,000 cc. with methyl alcohol. Stand 3–4 days before using.

Phosphate buffer solution: Dissolve 71.64 g. $Na_2HPO_4 \cdot 12H_2O$ in dist. water and make up to 1,000 cc.

To prepare the buffer mixture for staining, add to 300 cc. dist. water 11–12 cc. citric sol., and 9–8 cc. phosphate soln. Determine for each batch of dry stain the precise amounts of the 2 buffer solns., which should total 20 cc., by mixing into each of 7 Coplin jars: dist. water, 30 cc.; stock stain, 2 cc.; acetone, 3 cc.; methyl alcohol, 3 cc.; citric buffer, 1.30, 1.25, 1.20, 1.15, 1.10, 1.05, 1.00 cc.; phosphate buffer, 0.70, .75, .80, .85, .90, .95, 1.00 cc.

Run sections down to water and stain 30–60 min. in following: stock stain, 2 cc.; acetone, 3 cc.; methyl alcohol, 3 cc.; stock buffer, 35 cc. Rinse, dehydrate in 2–3 changes of acetone; pass thru acetone and xylene, 1:1; clear in 2–3 changes of xylene and mount in liquid petrolatum. Seal with pyroxylin cement. This technic has given best results with formalin fixed material in routine surgical and post-mortem diagnosis.—*J. A. de Tomasi.*

PFUHL, W. and DIENSTBACH, O. **Die Speicherung und Verarbeitung von kolloiden Farbstoffen, Pigmenten und Lipoiden in der Golgi-Substanz der Leberzellen.** *Zts. Anat. u. Entwgesch.*, **108,** 260–82. 1938.

Trypan blue, lithium carmine, and collargol, when injected as intravital stains, were taken up by liver cells to a different degree in different species. In rabbits and guinea pigs the uptake was rapid and strong; in mice and rats it was much less; while in dogs the cells were refractory. The staining was fully developed in 1 to 2 days and faded slowly thereafter. India ink was taken up only by the star cells. The doses of stains were: 300-g. guinea pigs, 6 cc. of 1% trypan blue solution subcutaneously; rabbits, per Kg., 4 cc. of 1% trypan blue or 4 cc. of 5% lithium carmine intravenously. For studies with collargol, young 800-g. rabbits were used to avoid the presence of natural pigment in the liver cells, and an intravenous injection of 2.5 cc. of 0.6% solution given. With moderate staining, the dye was taken into the Golgi apparatus of the cells in the form of granules, but with much larger quantities of dye and consequently stronger staining, the entire Golgi apparatus was stained. The dye was seen in the same locations as natural pigment and lipoids. The authors conclude that a pathway of execretory process is represented by the course of the dye in and out of the cells, but that it is unwise to generalize on its behavior from animals to man or from one species of animal to another.—*H. A. Davenport.*

SHANNON, J. A. **The renal excretion of phenol red by the aglomerular fishes, Opsanus tau and Lophius piscatorius.** *J. Cellular & Comp. Physiol.*, **11,** 315. 1938.

The study consists of observations on the renal excretion of phenol red in the aglomerular fishes, *Lophius piscatorius* and *Opsanus tau*. The data indicate that the essential limitation in tubular excretion of phenol red in these kidneys is the circumstance that the tubules can transfer to the urine only a certain maximal quantity of dye per unit time, as in the glomerular kidneys of the chicken, dog and man.

The fact that phenol red may still be excreted at this maximal rate even tho the concentration of free dye in the plasma is higher than in the urine, indicates that the renal tubule is impermeable to phenol red in the sense that diffusion does not occur across the tubule. Only thru the normal mechanism of excretion can phenol red gain access to the tubule lumen.—*L. Farber.*

PLANT MICROTECHNIC

BANK, O. **Die Vitalfärbung des Zellkernes mit basischen Farbstoffen.** *Protoplasma*, **29,** 587–94. 1938.

The author reviews his previous papers on methods for vital staining of the nucleus in the epidermis cells of *Allium cepa*. He concludes that the official

differentiation of structure is a necessary corollary to the stainability of the nucleus. This he regards as a dehydration process which he secured by immersing the tissue for 3–20 min. in a 0.1% solution of the dye containing 1% of anilin. He also used alcohol, ether, chloroform and oleic acid, but the best agent proved to be anilin. The dyes which thus give vital staining are methylene blue, neutral red, toluidine blue, brilliant cresyl blue, Janus green B, malachite green, Bismark brown and safranin (all presumably of Grübler). The nuclear structure is reversible by exposing the cells to plasmolytic agents, 2 mol $CaCl_2$ or $Ca(NO_3)_2$ in relation to the dye solution of 1:1 in which mixture the nucleus becomes homogeneous and diffusely colored. With prune pure (a basic dye) he obtained nuclear staining only by immersing the cells in the dye and anilin solution which, from the beginning, contained 1 mol $Ca(NO_3)_2$. This solution was slightly alkaline. With eosin (an acid dye) he obtained staining in 0.1% eosin or erythrosin plus 1 mol $Ca(NO_3)_2$ in proportions of 1:1 containing 0.4% acetic acid.—*R. Chambers.*

KUSTER, E. **Über Vakuolenkontraktion und Membranfärbung bei Helodea nach Behandlung mit Vitalfärbemitteln.** *Zts. Wiss. Mikr.*, **54,** 433–44. 1938.

Vital staining was used to examine the causes of contraction of vacuoles, also to note the staining of membranes. Leaves of *Elodea densa* and of *E. canadensis* (collected in Giessen) were immersed in neutral red solutions of 1:1000, 1:5000, and 1:10,000 in dist. water and in tap water. The cell sap became definitely red in either solution. Vacuolar contraction took place more readily in *E. densa* than in *E. canadensis*. The greatest contraction occured in the peripheral cells with a decreasing gradient toward the center; the cells of the lower surface manifested the contraction more readily than those of the upper surface; but considerable variability in these reactions was noted. At times a deeply stained portion of the vacuole was separated from the remainder of the vacuole.

A temperature increase to 30° C, wounding, narcotics (2 cc. ether and 98 cc. tap water and neutral red), and some poisons (0.5% Na dehydrocholate and neutral red) accelerated the vacuolar contraction. Usually the cell walls were deeply stained when the solution was made up with dist. water, but took no stain when tap water (pH 7.2) was used.

Methylene blue, thionin, and Nile blue sulfate produced spotty stains in the cell walls, which may be correlated with manganese deposits in *Elodea* as well as in *Hydrilla.*—*Thora M. Plitt.*

PEKAREK, J. **Über die Wirkung von Nitraten auf die Färbung pflanzlicher Zellmembranen und Zellsäfte mit Azur I.** *Protoplasma*, **30,** 161–85. 1938.

Azure I (obtained in 1928 from Dr. G. Grübler and Co.) was used in 0.01, 0.006, 0.003 and 0.001% concentrations in tap water (pH about 7.5) on epidermis cells of the onion scales of *Allium cepa*. The dye colors intensely the cell walls and the sap. Placing the stained tissue in a hypo or isotonic solution of KNO_3 (0.2–0.25 mol.) causes gradual, diffuse decoloration of the cell walls. When hypertonic solutions of KNO_3 are used, viz., 0.3, 0.5, and 1 0 mol, the decoloration of the walls is accompanied by a precipitation of the dye between the cellulose walls and the protoplast. The shape and size of the precipitation granules and crystals depend on the length of exposure to and strength of the KNO_3 solution. With overstained preparations the salt will cause precipitation of crystals also in the sap.—*R. Chambers.*

STRUGGER, S. **Die Vitalfärbung des Protoplasms mit Rhodamin B und 6G.** *Protoplasma*, **30,** 85–100. 1938.

Rhodamin B (Merck) and rhodamin 6G (I.G.) in tap water (1:1000) vitally stain the protoplast of onion scale epidermal cells without any color accumulating in the vacuole. The staining is best appreciated by fluorescence with ultraviolet light. With rhodamin B the cytoplasm is diffusely colored, the chondriosomes especially are highly fluorescent. Variations in pH of the medium (with phosphate buffers) from 5.0–10.0 show no appreciable difference, hence his conclusion that the dye penetrates and accumulates on chondriosomes by virtue of its lipoid solubility. Rhodamin 6G is more toxic; otherwise it is similar to rhodamin B except that it vitally stains the nuclear network, while rhodamin B stains only the nuclear membrane.—*R. Chambers.*

TRUE, R. P. **Gall development on Pinus sylvestris by the Woodgate Peridermium, and morphology of the parasite.** *Phytopathology*, **28**, 24–49. 1938.

To differentiate mycelium of *Peridermium* in host tissues, orseillin BB in 3% aq. solution of acetic acid was used, also with anilin blue in a 3% solution of acetic acid, or a sat. solution of crystal violet in clove oil as a counterstain. The latter alone brought out the hyphae and haustoria well.—*S. C. Bauser.*

WATKINS, G. M. **Histology of Phymatotrichum root rot of field grown cotton.** *Phytopathology*, **28**, 195–202. 1938.

Hyphae of *Phymatotrichum* in sections of cotton roots, fixed in lactophenol, were well differentiated in periderm, phloem and cambium by cotton blue in lactophenol: treat root sections in lactophenol 15 min. or more, transfer to a 0.5% solution of cotton blue in lactophenol 5–15 min., wash in lactophenol and mount. —*S. C. Bauser.*

MICROÖRGANISMS

GLASS, V. and TABET, F. **A comparison of brilliant green-eosin and bismuth agar in the isolation of Bact. paratyphosum B from faeces.** *J. Path. & Bact.*, **46**, 195–8. 1938.

The brilliant-green-eosin medium (Jones, E. R., J. Path. & Bact., **42**, 455. 1936) demonstrates slightly more *Bact. paratyphosum B* organisms and inhibits *B. coli* considerably more than the bismuth medium. Paratyphoid colonies, however, are easier to detect on bismuth agar. Certain lactose fermenters give trouble in the dye medium but not in the bismuth medium.—*S. H. Hutner.*

KNAYSI, G. and FORD, M. **A method of counting viable bacteria in milk by means of the microscope.** *J. Dairy Sci.*, **21**, 129–41. 1938.

A direct microscopic method for distinguishing dead from living bacterial cells in milk is described. One ml. of a well-mixed sample of milk is measured into each of 2 clean test tubes, A and B. To each tube is added 0.5 ml. of a dye mixture containing equal parts of 0.2% methylene blue and 0.2% Nile blue sulfate. Contents are well mixed. To one tube, say B, a drop of 0.7–0.8 *N* NaOH is added and mixed thoroly. Both tubes are warmed to 45–50° C. To each tube 0.5 ml. of melted hardening mixture (2.0% agar and 8.0% gelatin), held at 45–50° C., is added and mixed. With Breed pipet, 0.01 ml. from tube A is placed on the center of right half of a clean glass slide. This is covered immediately with a clean 1.28 x 1.28 cm. cover glass. The droplet spreads and forms a film. Do likewise with tube B on left half of slide. The films are allowed to harden at room temp. or in refrigerator for about 2 min. The blue stained cells from tube A represent the dead cells originally present in milk, those in tube B the total bacterial content of the milk. The difference represents the viable cells. Ordinarily it is not necessary to remove fat, but if it is the milk is warmed to 35° C., heptane is added, the mixture shaken vigorously for 3–5 min. and centrifuged. The fat-heptane layer is then removed.—*H. Macy.*

RITTER, C. **Revised fuchsin lactose broth for the confirmatory test in water analysis.** *Amer. J. Pub. Health*, **28**, 616–20. 1938.

Tests on samples of water and on pure cultures show that the content of basic fuchsin in fuchsin lactose broth should be increased from 0.015% to 0.025% when this broth is used as a confirmatory medium, in order to decrease the number of false positives. The spurious tests are caused by streptococci, *Cl. welchii*, and other forms. Evidence is also presented which indicates that fuchsin lactose broth containing 1% bile and 0.1% fuchsin is a satisfactory confirmatory medium. —*M. W. Jennison.*

STAINS RECENTLY CERTIFIED

In the table below is given a list of the batches of stains approved since the last one listed in the July number of this Journal.

STAINS CERTIFIED JUNE 1, 1938 TO AUG. 31, 1938*

Name of dye	Certification No. of batch	Dye Content	Objects of tests made by Commission†	Date approved
Giemsa stain	NGe—3	——	As blood stain	June 3, 1938
Giemsa stain	GGe—5	——	As blood stain	June 3, 1938
Methyl green	NG—13	76%	In histology and as constituent of Pappenheim stain	June 4, 1938
Eosin Y	NE—13	91%	In histology and as constituent of blood stain	June 13, 1938
Methylene blue	NA—13	88%	As histological and bacteriological stain; and as constituent of blood stain	June 30, 1938
Orange G	LO—4	74%	As histological counterstain	July 14, 1938
Methyl violet	LMv—2	80%	As histological and bacteriological stain	July 14, 1938
Anilin blue WS	LK—2	——	As histological stain	July 15, 1938
Ethyl eosin	NEe—5	77%	For staining Negri bodies	Aug. 1, 1938
Eosin Y	LE—12	89%	In histology and as constituent of blood stain	Aug. 2, 1938
Orange G	NO—7	87%	As histological counterstain	Aug. 31, 1938

*The name of the company submitting any one of these dyes will be furnished on request.

†It is not to be inferred that these are the only uses for which each of these samples may be employed. The Commission ordinarily tests each dye for such of its common uses as seem to give the most severe check as to its staining value. Certification does not in any instance, however, imply approval for medicinal use.

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